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Sex steroids of black scabbardfish, *Aphanopus carbo*, in relation to reproductive and migratory dynamics

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ABSTRACT

Black scabbardfish, *Aphanopus carbo*, is a commercially important species that takes distant migrations throughout its life cycle. Sex steroids were measured by radioimmunoassay in the blood plasma of specimens caught off the Madeira Archipelago and mainland Portugal to link this species migratory path with its reproductive cycle. Furthermore, a pilot study using Mozambique tilapia (*Oreochromis mossambicus*) was designed to evaluate the effect of sample freshness on steroid levels because black scabbardfish blood was collected at separate times after specimens were caught. The changes in T and 11-KT concentrations between the time of blood extraction and the time after preservation did not statistically differ among the different methods applied. Therefore, measured black scabbardfish steroid concentrations were directly used in the subsequent data analyses. In females, E2 and in T concentrations peaked at a late stage of vitellogenesis. E2 concentration was significantly different between females caught off each area. Clustering E2 and T concentrations from all developing females resulted in the separation of two distinct groups, independently of their geographical area. In males, T and 11-KT were not significantly different between maturity stages. The hepatosomatic index of males caught off mainland Portugal was relatively high. This may reflect a mechanism for storing energy that will later be consumed during migration to the spawning grounds. The trend of sex steroids concentrations throughout the sexual maturation of the species is consistent with the morphological indicators and shows evidence of the reproductive and migratory pattern hypothesised for the black scabbardfish in NE Atlantic.

1. Introduction

The black scabbardfish, *Aphanopus carbo* Lowe, 1839, is an important commercial deep-sea species caught in Portuguese waters. This benthopelagic teleost fish is widely distributed along the NE Atlantic (Allain, Biseau, & Kergoat, 2003; Bordalo-Machado et al., 2009; Ehrich, 1983), where it undergoes large-scale migrations: the smallest specimens are reported further north and the largest specimens at the southernmost limit of distribution (Farias, Morales-Nin, Lorance, & Figueiredo, 2013; Ribeiro Santos, Minto, Connolly, & Rogan, 2013). The only known spawning grounds are located off Madeira, the Canary Islands, and possibly Morocco (Figueiredo et al., 2003; Pajuelo et al., 2008; Perera, 2008, p. 71). Juveniles recruit to the fisheries off the west of the British Isles, documented to be a feeding area, where they remain some time growing (Figueiredo et al., 2003; Ribeiro Santos, Minto, et al., 2013). Black scabbardfish subsequently moves to areas off mainland Portugal where caught specimens attain larger sizes and pre-spawning individuals are seldom captured (Figueiredo et al., 2003; Neves et al., 2009). After spending some time here to feed and grow, juveniles and pre-adults move further south to the spawning areas around Madeira (Farias et al., 2013). A key question is why fishes off mainland Portugal do not develop beyond the pre-spawning stage, despite attaining sizes larger than the estimated length at first maturity (L_{50} = 102.8 cm) (Figueiredo et al., 2003).

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Fish diet, growth, reproduction, and energy consumption are closely dependent on environmental conditions, which highly constrain metabolic rate processes in the deep-sea (Merrett & Haedrich, 1997, p. 282). Understanding the role of hormonal regulation of deep-sea species' vital processes will contribute to a better knowledge of their spatial dynamics. In this context, associating alterations in gonad development with the levels of sex steroids in blood plasma has proven to be a valuable tool to comprehend the endocrine control of reproduction in some deep-sea teleosts although this is a field not fully explored (Lee & Yang, 2002; Pankhurst & Conroy, 1987; Sequeira et al., 2017; Sisneros, Forlano, Knapp, & Bass, 2004).

In teleost fish females, the follicle stimulating hormone (FSH) is responsible for the stimulation of granulosa cells to produce estradiol-17β (E_2), the key hormone that induces the liver to synthesize vitellogenin and egg shell proteins, which are incorporated into the oocyte during vitellogenesis (Lubzens, Young, Bobe, & Cerdà, 2010). After the growth phase, a surge of luteinizing hormone (LH) stimulates the follicle to produce the maturation-inducing steroid − either 17,20β-dihydroxypregn-4-en-3-one (17,20β-P) or 17,20β,21-trihydroxypregn-4-en-3-one (17,20β,21-P), depending on the species – that promotes final oocyte maturation (resumption of meiosis) and ovulation (Lubzens et al., 2010; Nagahama & Yamashita, 2008).

In males, FSH regulates Sertoli cell activity to support germ cell development while LH acts on Leydig cells to promote steroidogenesis (Chauvigne, Zapater, Gasol, & Cerdà, 2014; Schulz, Sumpter, & Stacey, 2010). The key androgen in males is 11-ketotestosterone (11-KT) which promotes germ cell proliferation and maturation, as well as the development of secondary sexual characters and the mediation of reproductive behaviours (Borg, 1994; Schulz et al., 2010). In males, 17,20β-P is responsible for endorsing the initiation of meiosis, for stimulating spermiation, and for enhancing sperm motility (by alteration of the pH and fluidity of the seminal fluid) and can act as a pheromone, e.g. in goldfish (Scott, Sumpter, & Stacey, 2010). Finally, both male and female gonads produce testosterone (T) which is a precursor of E_2 and 11-KT and feeds back on the pituitary gland to promote the synthesis of gonadotrophins, among other functions (Lubzens et al., 2010; Nagahama, 1994; Schulz et al., 2010).

The main objective of this study was to assess and compare the hormonal status and gonadal development stage of specimens caught off mainland Portugal and off Madeira. Black scabbardfish specimens caught by commercial fisheries are already dead when pulled on board and it was only possible to obtain blood from specimens several hours after capture or thawed. To establish how these conditions may affect hormone levels, a pilot study tested the effect of sample age and freezing on the sex steroids of Mozambique tilapia, *Oreochromis mossambicus* (Peters, 1852).

2. Material and methods

2.1. Pilot study

To assess the effect of the time lag between death (capture) and blood collection and the effect of freezing (specimen storage) on sex steroid levels, Mozambique tilapia (*Oreochromis mossambicus*) male specimens were used. The Mozambique tilapia is a freshwater cichlid that can live at temperatures ranging from 8° to 42 °C and can be reared in hypersaline conditions (Froese & Pauly, 2019). Despite the biological and ecological differences between the Mozambique tilapia and the black scabbardfish, the former was used in this pilot study because specimens were readily available from a captive stock, raised from fertilised eggs at the University of Algarve and maintained in freshwater under natural annual conditions of photoperiod and water temperature (26 °C) prior to these experiment. Males were chosen over females because they are expected to show less hormonal variation owing to a simpler reproductive physiology.

Fish care and experimentation complied with the national legislation for the use of laboratory animals under a Group-1 license issued by the Portuguese National Authority for Animal Health. Fish were stunned and killed by immersion in iced water and total length (TL, cm) and total weight (TW, g) were measured. Blood samples were collected in heparinised syringes from the caudal peduncle in larger specimens or from the heart in smaller specimens.

Table 1
Experimental design and summary of Mozambique tilapia samples used in the pilot study. T, storage temperature; *n*, sample size; TL, total length range; TW, total weight range.

Treatment	T (°C)	Time (days)	*n*	TL (cm)	TW (g)
t1	4	1	6	15.2–18.9	61.74–103.62
t2	4	2	5	15.8–17.3	62.09–75.65
t4	4	4	6	16.5–18.9	66.4–105.21
t15	−20	15	6	15.1–19.3	53.7–103.52
t30	−20	30	5	14.6–17.2	50.68–81.64

After a first sample of blood was collected from 29 specimens (control, t0), fish were randomly separated into two groups: 18 fish were stored on ice in a refrigerator (temperature 4 °C) and the remaining 11 fish were frozen (temperature −20 °C). A second blood sample was collected from each fish at the distinct times that defined the treatments (Table 1). Frozen fish were thawed at room temperature prior to blood collection. Blood samples were centrifuged at 4 °C and separated plasma was stored at −20 °C until assay.

2.2. Black scabbardfish samples

Black scabbardfish (*Aphanopus carbo)* specimens were collected at irregular times between 2010 and 2012 from commercial longline vessels operating off Madeira Archipelago and off mainland Portugal (Table 2). Since *Aphanopus intermedius* Parin, 1983 (intermediate scabbardfish) specimens are mixed with *A. carbo* in Madeira's commercial landings (Stefanni & Knutsen, 2007), specimens caught in this region were assigned to species following the morphological criteria defined by Biscoito et al. (2011). In the present study, only *A. carbo* specimens were used.

In Madeira, specimens were sampled between October and December from commercial port landings. This month range was selected to encompass the reproductive season. In this fishery, black scabbardfish are caught by mid-water horizontal drifting longline set below 1000 m deep and the soaking time lasts from two to four days (Bordalo-Machado et al., 2009). Specimens from mainland Portugal were collected throughout the year on board commercial vessels or from commercial port landings. In this fishery, the fishing gear is a horizontal bottom longline and the soaking time lasts from one to two

Table 2
Summary of black scabbardfish samples used for measuring sex steroids estradiol-17β, testosterone, and 11-ketotestosterone. Values are total length range (mm) and sample size (parenthesis) by sex and maturity stage (1–5).

		Region	
Sex	Maturity	Madeira	Mainland Portugal
Females	1	-	890-1081 (13)
	2	1087-1506 (16)	1041-1178 (6)
	3	1141-1254 (4)	-
	4	1135-1323 (7)	-
	5	1141-1291 (4)	-
Males	1	-	874-1003 (10)
	2	1117-1179 (2)	1045 (1)
	3	1115-1177 (4)	-
	4	1122-1233 (7)	-
	5	1040-1244 (4)	-

days (Bordalo-Machado et al., 2009).

For each specimen, the following information was collected: total length (TL, mm), total weight (TW, g), gutted weight (UW, g), liver weight (LW, g), gonad weight (GW, g), sex, and maturity stage. Maturity stage was macroscopically assigned following the scale proposed by Gordo et al. (2000, p. 35): stage 1, immature or resting; stage 2, developing; stage 3, pre-spawning; stage 4, spawning; and stage 5, post-spawning or spent.

The gonadosomatic index (GSI) was calculated as the gonad weight as a proportion of the gutted weight; the hepatosomatic index (HSI) was calculated as the liver weight as a proportion of the gutted weight; and Fulton's condition factor (K) was calculated as the ratio between the gutted weight and the cube of the total length. To calculate the previous reproductive indicators, whole data sets collected between 2010 and 2012 from specimens caught off Madeira ($n = 1976$) and off mainland Portugal ($n = 692$) were used.

Blood samples were collected in heparinised syringes from the specimen's caudal vessel, which was exposed by removing the lateral musculature close to the caudal peduncle. To minimize the effect of metabolism and degradation, the collection of blood samples took place as soon after hauling as possible. In specimens caught off Madeira, blood was extracted in the laboratory from fish that had been dead and remained hooked to the longline for one to four days and was kept on ice after hauling for less than 24 h ($n = 48$). The exact individual time of death or hauling is not known since each vessel deploys the fishing gear more than once during each fishing trip and the fish is kept on board all together. Specimens caught off mainland Portugal had been dead and remained hooked to the longline for up to one day. Blood samples from these specimens were collected (i) on board from fresh fish immediately after hauling ($n = 12$); (ii) in the laboratory from fish kept on ice after hauling for less than 24 h ($n = 14$); or (iii) in the laboratory from thawed fish that was stored in a freezing room at −20 °C, less than one day after hauling, for 30 days ($n = 4$).

Blood samples were centrifuged at 4 °C to separate the plasma, which was stored at −20 °C until assay.

2.3. Steroid analysis

Blood plasma (100 μL) was extracted twice with 3 ml of diethyl ether to obtain the free steroids. Extracts were dried on a dry bath at 40 °C under nitrogen gas and suspended in 1 ml of assay buffer (0.5 M phosphate–gelatine buffer, pH 7.6). Free steroids were measured by radioimmunoassay (RIA) following the methodology described by Scott, Sheldrick, and Flint (1982). Individual plasma samples were mixed with 100 μL of distilled water and extracted twice with 4 mL of diethyl ether to obtain free steroids. Extracts were dried under nitrogen, reconstituted in 0.5 M phosphate–gelatine buffer, pH 7.6 and steroids were measured by RIA.

The estradiol-17β (E_2) antiserum was purchased from Research Diagnostics (USA) and the cross-reactions (%) have been reported as follows: < 0.2% for 4-pregnene-3,20-dione; < 0.2% for 11β,17,21-trihydroxy-4-pregnene-3,20-dione; < 0.2% for 4-androstene-3,17-dione; < 0.2% for 17β-hydroxy-4-androsten-3-one; < 0.2% for 3β-hydroxy-5-pregnen-20-one; < 0.2% for 3β-hydroxy-5-androsten-17-one; 15% for 3β-hydroxy-1,3,5(10)-estratrien-17-one; 8% for 3,17β-dihydroxy-1,3,5(10)-estratrien-16-one; 0.7% for 3,16α,17β-trihydroxy-1,3,5(10)-estratrien-3-one; < 0.2% for 3,16α-dihydroxy-1,3,5(10)-estratrien-17-one (Guerreiro, Fuentes, Canario, & Power, 2002). The testosterone antiserum was kindly donated by Dr. David Kime (University of Sheffield, UK). The testosterone (T) antiserum cross-reactions were 63% for androstenedione, 35% for 11-ketotestosterone, 55% for 11-hydroxytestosterone, 40% for 5-androstan-17-ol-3-one, 31% for 5-androstan-17-ol-3-one, 12% for 5-androstane-3,17-diol, 25% for 5-androstane-3,17-diol. The 11-ketotestosterone (11-KT) antiserum cross-reactions were 20.1% for 11-hydroxytestosterone, 20.6% for testosterone, 76.9% for androstenedione, 30.1% for 11-hydroxyandrostenedione, 52% for dihydrotestosterone, 3.3% for cortisol, and 1.3% for cortisone (Kime & Manning, 1982). All samples were assayed in duplicate in a single assay. The intra-assay and inter-assay coefficients of variation were, respectively: 6.6% and 14.2% for E_2; 5.0% and 8.2% for T; and 8.2% and 11.6% for 11-KT. The limits of detection were between 10 (E_2) and 100 (T and 11-KT) pg/mL.

2.4. Statistical analyses

In the Mozambique tilapia pilot study, one-way ANOVA was applied for comparing T and 11-KT concentrations between t0 (control) and the time of each treatment. The variation in T and 11-KT concentration in Mozambique tilapia was estimated as the difference between the value at time t0 (control) and at the time of each treatment. One-Way ANOVA was applied for comparing the variation in T and in 11-KT between treatments. Whenever necessary, data were $\log_{10}$-transformed to meet the ANOVA assumptions. If ANOVA assumptions were not met after data transformation, nonparametric Kruskal–Wallis test by ranks was used instead. When there were statistically significant differences between treatments, Wilcoxon signed-rank test was applied to compare each pair of treatments.

In black scabbardfish blood plasma, one-way ANOVA was used to investigate the association between E_2, T, and 11-KT concentrations and the way the specimens are preserved prior to blood collection for each sex separately. Whenever necessary, data were $\log_{10}$-transformed to meet the ANOVA assumptions. If assumptions were not met after data transformation, nonparametric Wilcoxon signed-rank test was used instead. When there were statistically significant differences between preservation methods, Wilcoxon test was applied to compare each pair of maturity stages.

Two-way fixed effects ANOVA was applied for analysing the effects of geographical region and maturity stage on GSI, HSI, K, and on E_2, T, and 11-KT concentrations for each sex separately. Whenever necessary, data were $\log_{10}$-transformed to meet the ANOVA assumptions. If assumptions were not met after data transformation, nonparametric Wilcoxon signed-rank test was used instead. When there were statistically significant differences between maturity stages, Wilcoxon test was applied to compare each pair of maturity stages.

A k-means clustering method was applied to steroid (E_2 and T) profile data using developing (stage 2) females both from Madeira and mainland Portugal using the R package *cluster* (Maechler, Rousseeuw, Struyf, Hubert, & Hornik, 2018, pp. 7–1).

The R software (R Core Team, 2018) was used for all statistical analyses and 5% significance level was adopted. Graphics were built with the R package *ggplot2* (Wickham, 2009).

3. Results

3.1. Pilot study

In Mozambique tilapia, no significant differences in T concentration (χ^2 (4, $n = 28$) = 3.918, *p-value* = 0.417) and in 11-KT concentration (χ^2 (4, $n = 28$) = 1.601, *p-value* = 0.809) were found between samples collected initially and samples collected after treatment (Fig. 1).

T and 11-KT concentrations varied between the control (t0) and each treatment but the variations were not significantly different between treatments (χ^2 (4, $n = 28$) = 3.918, *p-value* = 0.417 and χ^2 (4, $n = 28$) = 1.601, *p-value* = 0.809, respectively) (Fig. 2).

3.2. Reproductive indicators in black scabbardfish

Black scabbardfish specimens caught off mainland Portugal, between 2010 and 2012, were in maturity stages 1–3 and 5 (Table 2). Immature or resting females and males (maturity stage 1) were not found among fish sampled in Madeira.

In females caught off mainland Portugal, mean GSI significantly

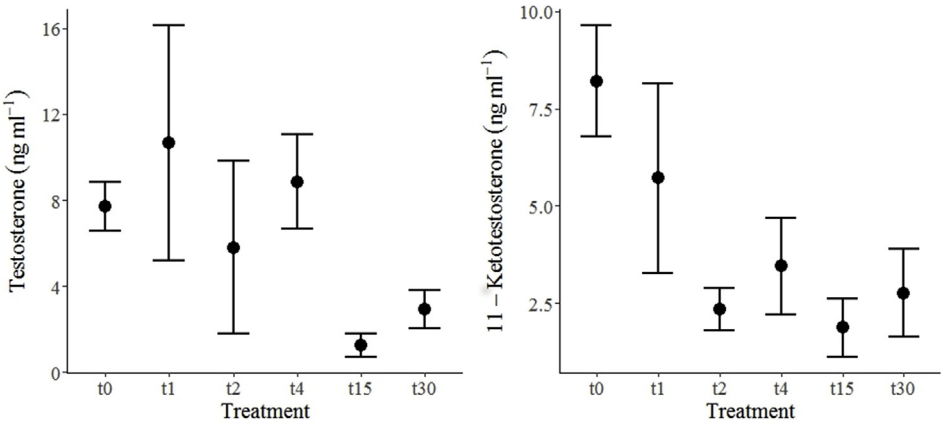

Fig. 1. Testosterone (ng ml^{-1}) (left) and 11-ketotestosterone (ng ml^{-1}) (right) concentration (mean ± SE) in Mozambique tilapia males by treatment.

increased between stage 1 and stage 2 (W = 3544, *p-value* < 0.001). However, the increase between stage 2 and stage 3 was not statistically significant (W = 164, *p-value* = 0.358) (Fig. 3). The mean GSI of females caught off Madeira significantly increased from stage 2 to stage 3 (W = 1866, *p-value* < 0.001) and from stage 3 to stage 4 (W = 1015, *p-value* < 0.001), and significantly decreased from stage 4 to stage 5 (W = 6983, *p-value* < 0.001). Concerning males, mean GSI significantly increased from stage 1 to stage 2 (W = 488, *p-value* < 0.001) and from stage 2 to stage 3 (W = 845, *p-value* < 0.001) in specimens caught off mainland Portugal, and from stage 2 to stage 3 (W = 17,335, *p-value* < 0.001) and from stage 3 to stage 4 (W = 2494, *p-value* < 0.001) in specimens caught off Madeira, whereas the decrease from stage 4 to stage 5 was statistically significant (W = 8213, *p-value* < 0.001) in specimens from Madeira. The mean GSI was significantly higher in specimens caught off Madeira than off mainland Portugal for stage 2 (W = 23,842, *p-value* < 2.2×10^{-16}) and stage 3 females (W = 7, *p-value* = 0.004) and for stage 2 (W = 11,430, *p-value* = 0.008) and stage 3 males (W = 8147, *p-value* = 0.033).

Mean HSI was significantly higher in stage 2 females caught off Madeira than off mainland Portugal (W = 33,667, *p-value* = 0.020) (Fig. 4). Mean HSI significantly increased from stage 1 to stage 2 females caught off mainland Portugal (W = 10,395, *p-value* < 0.001) and from stage 2 to stage 3 females caught off Madeira (W = 13,287, *p-value* < 0.001), whereas it significantly decreased from stage 4 to stage 5 females caught off Madeira (W = 5690, *p-value* < 0.001).

Mean K significantly increased between stage 1 and stage 2 females (W = 9041, *p-value* < 0.001) and males (W = 7505.5, *p-value* = 0.002) caught off mainland Portugal, and from stage 3 to stage 4 males (W = 7946, *p-value* < 0.001) caught off Madeira, whereas it significantly decreased from stage 4 to stage 5 males (W = 5828, *p-value* < 0.001) (Fig. 5). Mean K was significantly higher in stage 2 females (W = 57,444, *p-value* < 0.001), in stage 2 males (W = 18,945, *p-value* < 0.001), and in stage 3 males (W = 15,444, *p-value* < 0.001) caught off Madeira than in the same sex and same maturity stage specimens caught off mainland Portugal.

3.3. Sex steroids in black scabbardfish

The changes of both T and 11-KT concentrations in Mozambique tilapia between the time of blood extraction and the time of measurement after preservation did not statistically differ among the different treatments applied. Therefore, the sex steroid concentrations quantified in the black scabbardfish samples were directly used in the data analyses.

In black scabbardfish specimens, no significant differences were found between the specimens preservation methods for all three analysed sex steroids: E_2 ($F_{2,41}$ = 2.851, *p-value* = 0.069); T ($F_{2,64}$ = 1.789, *p-value* = 0.175); and 11-KT ($F_{1,26}$ = 0.239, *p-value* = 0.629).

In black scabbardfish females, T was not significantly different between regions ($F_{1,36}$ = 1.470, *p-value* = 0.233) nor between maturity stages ($F_{1,36}$ = 0.830, *p-value* = 0.368) neither was the interaction between region and maturity stage statistically significant ($F_{1,36}$ = 1.349, *p-value* = 0.253) (Fig. 6). E_2 was significantly different between regions ($F_{1,40}$ = 5.717, *p-value* = 0.022), but no significant

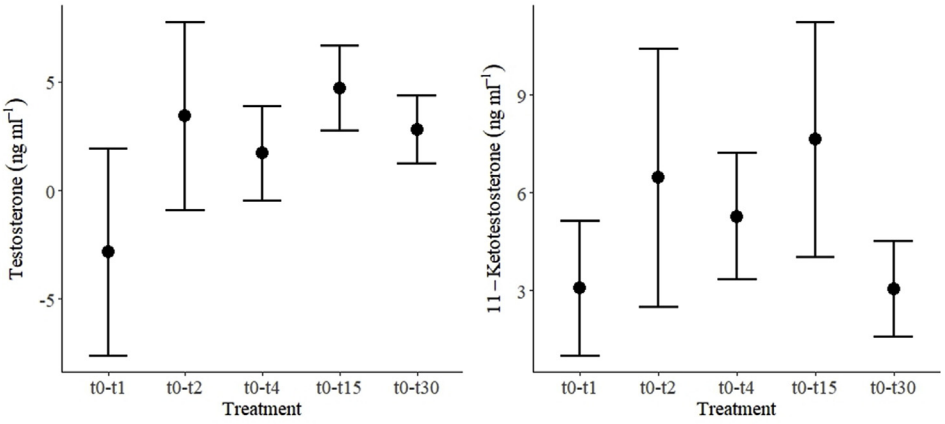

Fig. 2. Change in testosterone (ng/mL) (left) and 11-ketotestosterone (ng/mL) (right) concentration (mean ± SE) in Mozambique tilapia males between time of blood collection and the time of the second collection defined by the treatment.

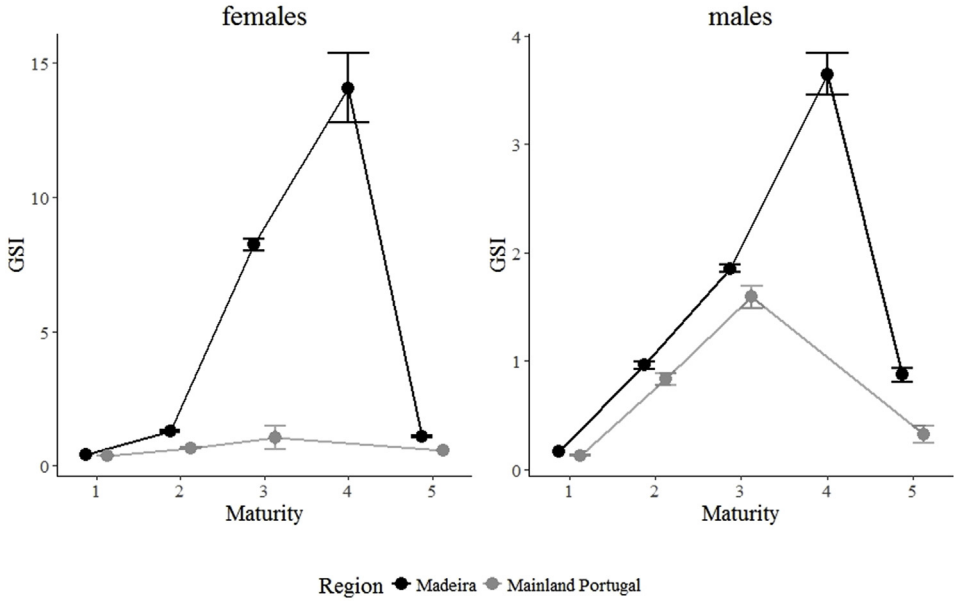

Fig. 3. Black scabbardfish gonadosomatic index (GSI) (mean ± SE) of females (left) and males (right) caught off Madeira and mainland Portugal by maturity stage.

differences were found between maturity stages ($F_{1,40}$ = 2.215, *p-value* = 0.145) neither was the interaction between region and maturity stage statistically significant ($F_{1,40}$ = 2.954, *p-value* = 0.093).

11-KT was significantly different between males caught off Madeira and males caught off mainland Portugal ($F_{1,24}$ = 14.364, *p-value* = 0.001), but no significant differences were found between maturity stages ($F_{1,24}$ = 0.465, *p-value* = 0.502) neither was the interaction between region and maturity stage statistically significant ($F_{1,24}$ = 0.389, *p-value* = 0.539) (Fig. 5). T in males was not significantly different between regions ($F_{1,24}$ = 2.228, *p-value* = 0.149) and no significant differences were found between maturity stages ($F_{1,24}$ = 0.008, *p-value* = 0.928). The interaction between factors was not analysed because factors were not crossed.

The only maturity stage within the same sex for which blood was collected in specimens caught off both Madeira and mainland Portugal areas was stage 2 females. Considering T and E_2 concentrations together, stage 2 female samples were grouped into two clusters that were statistically different ($F_{1,16}$ = 6.793, *p-value* = 0.019). T and E_2 concentrations did not show evidence of geographic differentiation as each cluster included specimens from both regions (Fig. 7).

4. Discussion

The pilot study with the Mozambique tilapia showed that it is possible to obtain meaningful measures of sex steroids in blood collected from fish that has been preserved refrigerated or frozen for a relatively prolonged period. Furthermore, it was assumed that the general behaviour of the hormonal steroids during refrigeration and the degradation mechanisms would follow similar patterns, although this is an area that requires more in-depth studies. In a previous study with plainfin midshipman fish, *Porichthys notatus*, blood plasma, no significant differences in E2, T, and 11-KT concentrations were found between samples collected at different times after capture (within one or 4 h) in either offshore or intertidal zones for both males and females

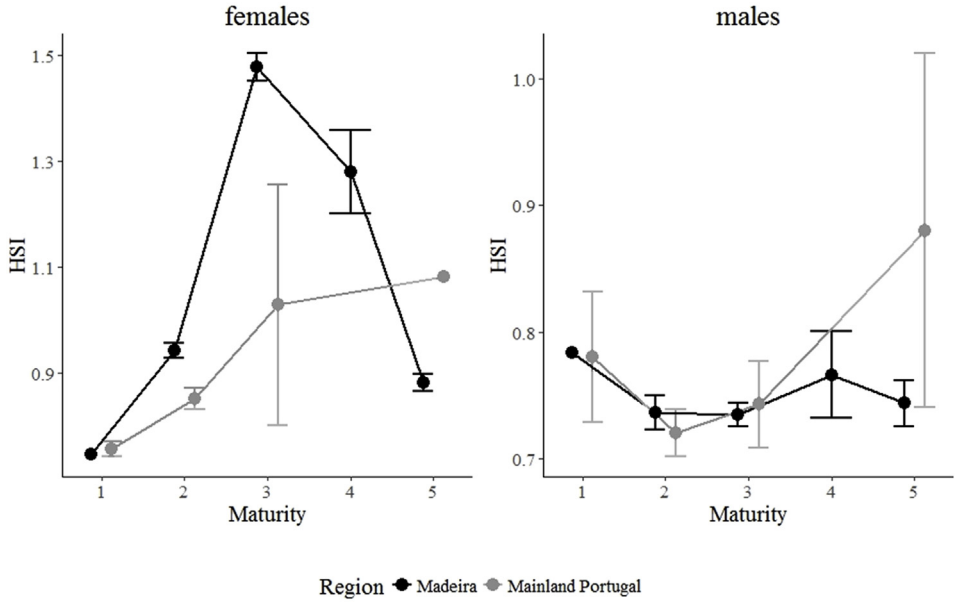

Fig. 4. Black scabbardfish hepatosomatic index (HSI) (mean ± SE) of females (left) and males (right) caught off Madeira and mainland Portugal by maturity stage.

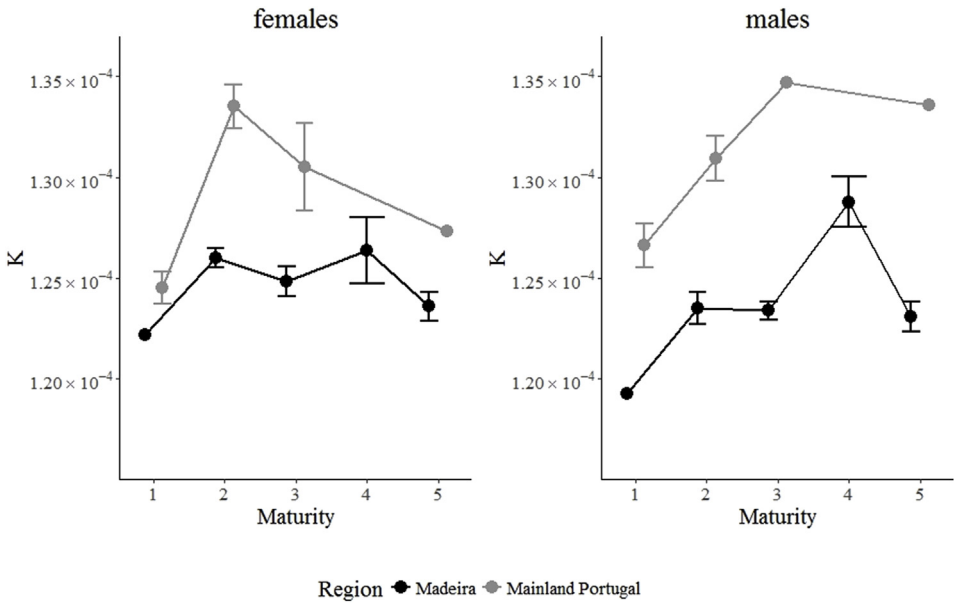

Fig. 5. Black scabbardfish Fulton's condition factor (K) (mean ± SE) of females (left) and males (right) caught off Madeira and mainland Portugal by maturity stage.

(Sisneros et al., 2004). Black scabbardfish blood samples were collected up to 24 h or 30 days after capture and steroid levels were not corrected. Nonetheless, black scabbardfish sex steroid levels should be regarded as relative and not absolute values. In addition to the time between death and blood collection and the above-mentioned factors that influence hormone levels, the stress of capture, which may vary with time and method of capture, can also lower sex steroid levels (Clearwater & Pankhurst, 1997; Cleary, Battaglene, & Pankhurst, 2002; Pankhurst & Conroy, 1988).

E_2 and T increased during vitellogenesis (stages 1–3), peaked at the

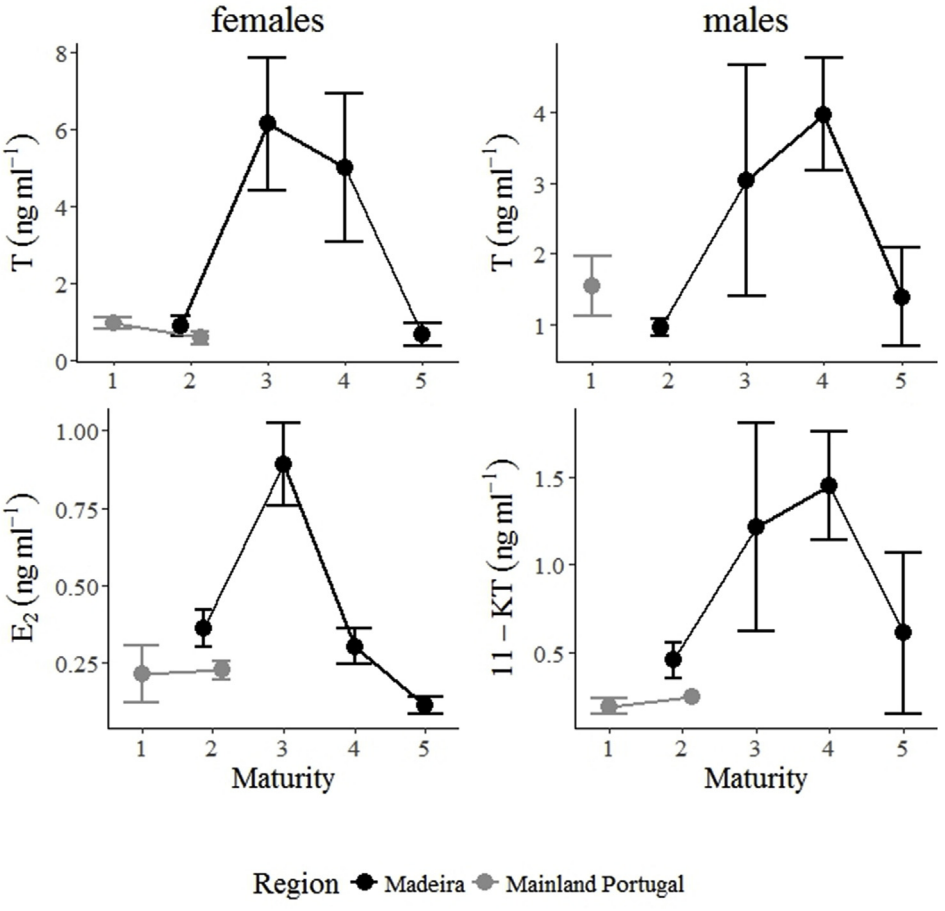

Fig. 6. Sex steroids concentration (mean ± SE) in the blood of black scabbardfish females (left column) and males (right column) caught off Madeira and mainland Portugal by maturity stage. T is testosterone in ng/mL; E_2 is estradiol in ng/mL; 11-KT is 11-ketotestosterone in ng/mL.

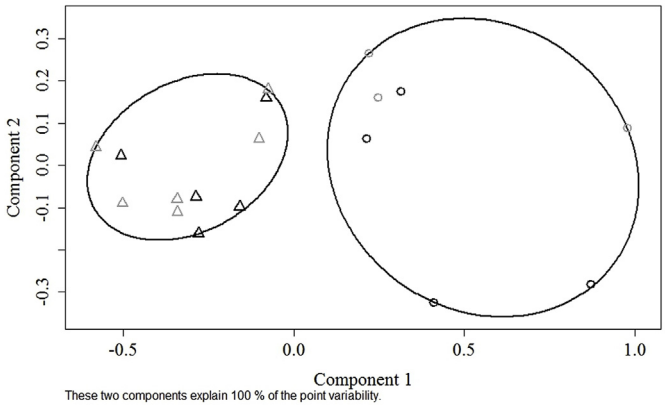

Fig. 7. Representation of k-means clustering applied to steroid (E_2 and T) profile data using developing (stage 2) females caught off Madeira and mainland Portugal.

pre-spawning stage (stage 3), and decreased from stage 3 onwards. The same steroid profile was observed for GSI in females. Although the increase in GSI between stages 1 and 2 was not statistically significant, the concomitance between the highest levels of E_2 and T and the peak in vitellogenesis (stage 3 in this species) has also been described for other teleosts (e.g. Frisch, McCormick, & Pankhurst, 2007; Hachero-Cruzado et al., 2007; Li, Liu, & Lin, 2007; Prat, Zanuy, Carrillo, de Mones, & Fostier, 1990). Furthermore, the same profile has been observed in the HSI of female black scabbardfish, demonstrating that the hepatic reserves are being consumed during the maturation process (Domínguez-Petit & Saborido-Rey, 2010; Ribeiro Santos, Minto, et al., 2013).

In fact, E_2 and T are connected since T is a precursor of E_2, such as other steroids (Pankhurst & Conroy, 1987), and E_2 is crucial for the start of vitellogenesis because it promotes the synthesis of hepatic yolk precursors (vitellogenin) in a variety of teleost species and provides negative feedback to LH secretion (Lubzens et al., 2010; Sisneros et al., 2004; Tyler & Sumpter, 1996). Chemical and physiological changes occurring throughout female developing stage are of uttermost importance for comprehending the reproductive cycle of black scabbardfish and the differences between fish living off Madeira and fish living off the Portuguese mainland coast. To disentangle this moment in the species' life cycle, E_2 and T concentrations in developing females were analysed together through cluster analysis. The mixing of females from both areas within each cluster shows that the steroid profiles are similar between areas. Madeiran females were sampled at the spawning season and during this period it is likely that females between an early and a late development stage may concur. Fish from the group with the lowest steroid concentrations represent an earlier development stage. These fish will not have the time to mature and reach the spawning stage and will fail to spawn in the current season. It is not a case of skipped spawning, which refers to a mature fish that fails to spawn at a given year (Rideout & Tomkiewicz, 2011), but the drivers of both processes are similar. In black scabbardfish, the developing stage during early vitellogenesis proved to be the critical window for the decision to spawn or not to spawn (Neves et al., 2009; Skjæraasen et al., 2010). The energy saved not developing into mature stages is invested in growth and in increased fecundity, similar to what happens when fish skip spawning (Jørgensen, Ernande, Øyvind, & Dieckmann, 2006). The persistence of a high proportion of relatively immature individuals in a plentiful environment may allow them to grow fast before the next reproductive cycle drives energy towards the gonads (Alonso-Fernández & Saborido-Rey, 2012; Folkvord et al., 2014; Roff, 1983). Moreover, the simultaneous occurrence of two groups of developing females with similar length, steroid levels, and reproductive indicators points to a long duration of this maturity stage.

Stage 2 females caught off mainland Portugal during the spawning period show a high degree of follicular atresia in the ovaries and do not develop into mature stages (Neves et al., 2009). The abidance of fish at the same location after the maturation process is interrupted explains why ca. 25% of individuals off mainland Portugal are larger than L_{50} but immature (Figueiredo et al., 2003; Neves et al., 2009; Farias et al., 2013). This phenomenon was also observed off the west of the British Isles (Ribeiro Santos, Minto, et al., 2013). The capability of specimens to migrate to the spawning grounds will depend on the completion of high energy reserves (Merrett & Haedrich, 1997, p. 282; Ribeiro Santos, Minto, et al., 2013). In fact, the relatively high HSI observed in immature males caught off mainland Portugal suggests that energy is being stored in the liver to be spent when moving to the spawning grounds. Black scabbardfish's readiness for migration was also supported by the high content of arachidonic acid (ARA), which is demanded for long distance movements (Sargent, Bell, McEvoy, Tocher, & Estevez, 1999; Tocher, 2003), that was found in the muscle of specimens caught off mainland Portugal (Farias et al., 2014). Off the west of the British Isles, the species prepares for migration towards the south, between January and April, through an intense feeding activity on blue whiting (Ribeiro Santos, Trueman, Connolly, & Rogan, 2013).

Androgen (T and 11-KT) levels did not significantly differ among reproductive stages. Nevertheless, in males caught off Madeira, the two steroids were low in spent and regressed fish, increased during gonadal recrudescence, and peaked at the end of spermatogenesis as described in several studies (Pankhurst & Conroy, 1988; Prat et al., 1990). The significant differences between regions are expected to be a consequence of the unbalanced sampling amongst maturity stages.

The present work supports the role of sex steroids as intrinsic triggers for gonadal maturation and spawning in black scabbardfish. It also shows that it is possible to measure sex steroids in blood plasma that was collected late after death and relate the values with the dynamics of the species reproduction, overcoming some sampling constraints of deep-sea species. Given the fact that specimens used in this study were collected from commercial fisheries with a soak time greater than one day, the number of blood samples were limited because, as the time passes after capture, the blood extraction is more difficult. Moreover, the depth at which black scabbardfish specimens were collected implies that fish are boarded already dead and, subsequently, the collection of an adequate volume of blood is problematic. To overcome these difficulties, dedicated surveys are required, which in the case of deep-sea fishes are costly and do not take place in Portuguese waters. Nonetheless, the present results put in evidence that the RIA method is appropriate for the quantification of sex steroid concentration even with small blood volumes. The metabolization of sex steroids after capture was considered to be negligible or to have occurred equally in all individuals, as the relationships between hormones and reproductive stages were maintained.

Since spawning occurs during a relatively short period, for future work it would be important to have a monthly or more frequent coverage in the two regions to test if there are differences between maturity stages related with the time of the year when specimens are caught.

Author statement

Inês Farias performed conceptualization, methodology, investigation, formal analysis, writing - original draft, writing - review and editing, and visualization. Elsa Couto, Neide Lagarto, and João Delgado performed investigation. Adelino V.M. Canário performed conceptualization, methodology, formal analysis, writing - review and editing, and supervision. Ivone Figueiredo performed conceptualization, methodology, formal analysis, writing - review and editing, supervision, and project administration.

Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. All financial support came from public funding.

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